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Formation and Human Risk of Carcinogenic Heterocyclic Amines Formed from Natural Precursors in Meat

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Abstract

A group of heterocyclic amines that are mutagens and rodent carcinogens form when meat is cooked to medium and well-done states. The precursors of these compounds are natural meat components: creatinine, amino acids and sugars. Defined model systems of dry-heated precursors mimic the amounts and proportions of heterocyclic amines found in meat. Results from model systems and cooking experiments suggest ways to reduce their formation and, thus, to reduce human intake. Human cancer epidemiology studies related to consumption of well-done meat products are listed and compared.

Introduction

Diet has been one factor associated for many years with differing cancer rates worldwide.^{1,2} A biologically plausible factor in this association was the discovery in the 1970s of mutagenic activity, as detected by bacterial test systems, in meats cooked for human food.^{3,4} Finding bacterial mutagens in meats paralleled the well-known presence of mutagens in the smoke from cigarettes at that time.⁵

The original discovery of mutagenic substances in cooked meats was followed by demonstrations in many laboratories worldwide that the mutagens were formed during cooking and that the formation process was temperature dependent. A large range of foods were analyzed and it was determined that the cooked muscle meats were the major sources of extractable mutagenic activity in bacterial tests.⁶

The precursors responsible for the mutagens were identified when the chemical structures of the first compounds from cooked fish ^{7,8} and beef ^{9,10} were determined. These meat-derived mutagens were heterocyclic amines having an amino-imidazo structure suggesting that creatine or creatinine was involved in the muscle meat reactions.

Early work in adding creatine to meat before cooking showed it increased mutagenic activity.¹¹ Experiments relating creatine levels in fish, which varied over a range of 2.5-fold, showed mutagenic activity after cooking to be only approximately correlated.¹² A later study of cooked meat from 17 animal species also showed that creatine or creatinine levels do not explain differences in mutagenic activity. These results suggested that other components were also important for the mutagen levels in cooked meats. Other work showed free amino acids to be involved in the formation of mutagenic activity,¹³ but not amino acids from proteins.¹¹

Analysis of the specific mutagenic compounds formed during cooking shows that amino acids are important and changes in these can affect the amount and types of mutagens found in the cooked meat. Importantly, knowledge of the formation conditions does suggest ways to cook meat that greatly inhibit the formation and, thus, the human intake of carcinogenic heterocyclic amines.

Formation in Meats

Figure 1 shows the structures of creatine and 7 of the amino-imidazo heterocyclic amine mutagens pyrosynthesized from creatine and other small molecules, such as amino acids and glucose. It is easy to see that the N-methyl-amino-imidazo moiety could form intact from creatine, but the source of other rings are derived from other small molecules,

and their sources are not apparent from the reactants. These chemicals were isolated by following their mutagenic activity in *Salmonella*-based mutation tests during extraction and chromatographic purification.^{7-9, 14-16} A breakthrough in the analysis of heterocyclic amines in meats and model systems was made with the development of solid-phase extraction methods, enabling the extraction of the compounds, followed by analysis for specific heterocyclic amines by high performance liquid chromatography (HPLC), to be made for a reasonable cost.¹⁷

The temperature dependence of the formation of these compounds in beef patties cooked to 70°C, near the U.S. Department of Agriculture/Food Safety and Inspection Services recommended internal temperature of 71.1°C, is shown in Figure 2. The sum of the mass amounts of heterocyclic amine compounds identified per gram of cooked meat is shown for each of the four pan temperatures, indicating a direct correspondence between increased cooking temperature and heterocyclic amine content. (REF.) Heat flow simulations to understand heterocyclic amine formation during the pan-frying of beef patties were done by Tran, *et al.* These simulations accurately modeled the experimental temperature increases, meat cooking times, heterocyclic amine spatial distribution, and total amount of heterocyclic amines produced.¹⁸

Studies of the amounts of heterocyclic amines produced in foods as a result of regional cooking practices are reported for Great Britain,¹⁹ Sweden,^{20, 21} Switzerland,²² Spain,²³ Japan,²⁴ and the United States.^{25, 26} In most cases, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) tend to be the most mass-abundant heterocyclic amines. Their concentrations in cooked meats typically range from nearly undetectable levels (typically

0.1 ng/g) to tens of ng/g for MeIQx and up to a few hundreds of ng/g for PhIP, depending on the cooking method.

Pan residues and food flavors

A source of heterocyclic amines related to the meats themselves are pan residues and process flavors. Pan residues are sometimes consumed after being made into gravy and can be a source of heterocyclic amines equivalent to or greater than that of the meat itself.^{20, 21, 27}

Process flavors are commercially produced flavors derived from heated mixtures of proteins, fats and carbohydrates. These are added to foods in amounts up to a few percent by weight to improve the food's taste and color, and they can also be used as a base for soups. Because of their chemical complexity, specific sample preparation methods for heterocyclic amine analysis have been developed for these.²⁸⁻³⁰ Although most process flavors have undetectable levels of heterocyclic amines, some samples contain as much as 20 nanograms heterocyclic amines per gram of solid or liquid flavor.³¹⁻³³ However, since process flavors are consumed as only a tiny percentage of the human diet, the bulk of heterocyclic amine exposure is from well-cooked meats.

Model systems

Model systems to understand the formation of the mutagenic/carcinogenic heterocyclic amines were developed to help identify the foods and cooking conditions favoring their formation and to develop strategies to reduce their formation and, thus, the human intake. Defined model systems composed of creatine or creatinine, amino acids,

and sugars have been a good model for the trace level formation of these heterocyclic amines. Jägerstad *et al.* developed a system for heating components in diethylene glycol,¹¹ and the work was followed by many studies investigating heterocyclic amine precursors³⁴⁻³⁶ and kinetics³⁷ in a sealed-tube aqueous model. Reaction intermediates were identified that lead to the formation of PhIP.³⁸ It was shown that 37°C is warm enough to produce PhIP from a mixture of phenylalanine with creatinine and glucose or MeIQx from glycine with creatinine and glucose in aqueous buffers.^{39,40} No PhIP was found in a similar model system at room temperature for two weeks.³⁸ Another meat model system that produced heterocyclic amines was composed of boiled pork juice.⁴¹

Simple dry-heating of heterocyclic amine precursors also forms similar relative amounts and types of heterocyclic amines as are seen in cooked meats. Table 1 shows amino acid, creatine, and glucose content of beef muscle, chicken breast, and codfish. When these components are combined and heated for 30 min at 225°C, a family of heterocyclic amines is formed, and these vary with the mixture composition as shown in Figure 3. Two of the compounds, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), are most abundant in the codfish model, but the codfish model produces the lower amounts of MeIQx and 2-amino-(1,6-dimethylfuro[2,3-e])imidazole[4,5-b]pyridine (IFP). The model of chicken meat produces the largest amount of PhIP, as is shown in studies of the cooked chicken breast meat.⁴²

Examining the composition of the model systems in Table 1 shows that arginine, glutamic acid, leucine and phenylalanine are greatly reduced in codfish compared to the beef or chicken breast. Phenylalanine, a known precursor for PhIP, is highest in the

chicken model system, and PhIP is also known to be formed from tyrosine and isoleucine, also highest in chicken.³⁶ IFP is known to form from glutamic acid,⁴³ and the IFP formation follows the glutamic acid content of the three mixtures shown. Results in Table 1 fit with the general findings of heterocyclic amines in meats: that IQ and MeIQ are seldom detected in beef or chicken; MeIQx is about equal in beef and chicken; large amounts of PhIP can be formed in chicken which is over-cooked, but PhIP levels similar to those seen in beef are measured in chicken cooked in most households sampled.⁴⁴

Modifying cooking practices to reduce the formation of heterocyclic amines

As shown in Figure 2, the formation of heterocyclic amines is related to pan temperature when meat is cooked to the same final internal temperature. Surprisingly, the time needed to reach the 70°C internal temperature is about the same at 250°C (7 min) as at 160°C (9 min).⁴⁵ This is due to the limit of the slow heat transfer through the meat, suggesting that simply using lower pan temperatures is a practical way to reduce heterocyclic amine formation, without greatly increasing cooking time.

Flipping pan-fried beef patties over every minute, compared to turning the meat over at 5 minutes, and cooking at moderate pan temperatures until the target internal temperature of 70°C is reached, seems to be the most effective way to reduce heterocyclic amine content, while also avoiding under-cooking (defined as cooking to a final temperature below 70°C, the internal temperature needed to eliminate harmful bacteria).⁴⁵

Minor changes in recipes for preparing different meat dishes may provide a way of reducing the amount of heterocyclic amines formed. The addition of reaction inhibitors or inert substances can change the concentration of precursors and show an inhibiting effect.

Schemes for reducing mutagenic activity or the specific heterocyclic amine by adding substances to ground meat have been reported. Food additives such as soy flour or antioxidants,⁴⁶ or glucose or lactose,⁴⁷ were shown to lower mutagenic activity.

The heat and mass transport in meat during frying is very complex. Water is important for the transport of water-soluble precursors for formation of heterocyclic amines within the food. The transport of precursors from the inner parts of the food to the surface can be restricted by the addition of water-binding compounds, such as salt, soy protein, or starch to minced meat, thus reducing the formation of heterocyclic amines. Persson *et al.* showed a significant effect with the addition of sodium chloride/sodium tripolyphosphate.⁴⁸ Enzyme treatment with creatinase was used to reduce the available creatine in meat.⁴⁹

Heterocyclic amine formation can be affected by meat surface treatment. The application of a 7 component marinade to chicken breast meat before grilling can greatly decrease PhIP, although MeIQx is increased at the longest cooking time, probably due to sucrose in the marinade.⁵⁰ No change in heterocyclic amines was seen after marinating chicken in another study⁵¹, possibly due to differences in marinating or cooking conditions. Conversely, a heterocyclic amine reducing effect was seen when sugar was mixed with ground meat formed into patties before frying.⁴⁷ Experiments with eggs, bean cake and pork show that added sugar and soy sauce increases most of the heterocyclic amines.⁵²

A microwave pre-treatment method reduced the amount of heterocyclic amines formed during the frying of ground beef.⁵³ Beef patties received microwave pre-treatment for various times before frying. Microwave pre-treating for 2 minutes, then pouring off

the resulting liquid and frying at either 200°C or 250°C for 6 minutes per side, reduced heterocyclic amines. The liquid released by the microwave pre-treatment contained creatine, creatinine, amino acids, glucose, water, and fat, and discarding these precursors resulted in lower heterocyclic amines. The sum of the heterocyclic amines present decreased 3-fold following microwaving and frying at 200°C or 9-fold following microwaving and then frying at 250°C, compared to controls (non-microwave pre-treated beef patties fried under identical conditions).

Human Risk

Table 2 summarizes human studies that investigated the relationship between meat cooking doneness and cancers at various sites. In rodents the heterocyclic amines are multi-site carcinogens. The number of studies and number of human cancer sites with positive correlations with meat doneness strongly suggests that these compounds may be multi-site carcinogens in humans as well. Supporting these epidemiology studies is a result showing women have an increased cancer risk with increasing levels of PhIP-DNA adducts, and that the DNA adducts increase with a subject's preference for well-done meat.⁸²

Conclusion

There is a general consensus that human exposure to potent genotoxic heterocyclic amine carcinogens produced in meat during cooking is widespread. Understanding the parameters affecting their formation also shows us ways to avoid their formation. The demonstrated mutagenicity of these compounds in bacteria³, cells in culture,^{83, 84} and in mice,⁸⁵ support the many studies of carcinogenicity in mice⁸⁶ and rats.^{87, 88} Mechanistic data show that, even at low

doses, heterocyclic amines form DNA adducts in rodents, primates, and humans.⁸⁹⁻⁹¹ The majority of epidemiological studies generally support the hypothesis that meat cooking doneness increases risk for human cancers.

The goal of understanding and reducing cancer incidence is worthwhile. Here we describe conditions for both formation and reduction of carcinogenic heterocyclic amines in our diet. We also describe 30 human cancer epidemiology studies that relate consumption of well-done meat in our diet to various tumor sites. More than 80% of these studies show a positive correlation between cancer incidence and well-done meat consumption.

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Table 1: Concentration (mg/g meat wet weight) of free amino acids, creatine and glucose in three meats (From Pais et al,²⁷).

	Beef	Chicken breast	Cod Fish
<i>L</i> -Alanine	0.14	0.21	0.12
<i>L</i> -Arginine	1.07	1.19	0.03
<i>L</i> -Aspartic acid	0.02	0.13	0.01
<i>L</i> -Glutamic acid	0.09	0.23	0.02
<i>L</i> -Glycine	0.06	0.08	0.05
<i>L</i> -Histidine	0.14	0.18	0.03
<i>L</i> -Isoleucine	0.05	0.08	0.02
<i>L</i> -Leucine	0.07	0.13	0.02
<i>L</i> -Lysine	0.07	0.14	0.18
<i>L</i> -Methionine	0.06	0.08	0.04
<i>L</i> -Phenylalanine	0.05	0.08	0.01
<i>L</i> -Proline	0.10	0.10	0.14
<i>L</i> -Serine	0.05	0.12	0.02
<i>L</i> -Threonine	0.28	1.63	0.69
<i>L</i> -Tyrosine	0.06	0.10	0.03
<i>L</i> -Valine	0.06	0.10	0.04
Creatine	6.33	3.54	7.06
Glucose	7.03	0.47	0.21

Table 2: Human studies investigating cancer and well-done meat cooking.

Study	Response: Odd's Ratio (OR) or Relative Risk (RR)	Site	Number of Subjects, Age range	Study Type: case-control (CC) or prospective (P)
Han et al., 2004 ⁵⁴	OR= 2.38	Breast	635, Ng	CC
Dai et al., 2002 ⁵⁵	OR=1.92	Breast	3015, 25-64	CC
Zheng et al., 2002 ⁵⁶	OR=3.4	Breast	683, post menopause	CC
Balbi et al., 2001 ⁵⁷	Barbecued OR=2.66 Fried=no association	Bladder	720, 40-89	CC
Zheng et al., 2001 ⁵⁸	2.0	Breast	488, 55-69	CC
Delfino et al., 2000 ⁵⁹	No association	Breast	394, >39	CC
Sinha et al., 2000 ⁶⁰	OR=1.9	Breast	930, 56-67	CC
Zheng et al., 1998 ⁶¹	OR=4.6	Breast	930, 55-69	CC
Butler et al., 2003 ⁶²	OR=2.0	Colon	1658, 40-80	CC
Kampman et al., 1999 ⁶³	OR=1.4, men only	Colon	3402, 30-79	CC
Sinha et al., 1999 ⁶⁴	OR=1.85/10g meat	Colon	374, Ng	CC
Augustsson et al., 1999 ⁶⁵	No association	Colon Rectum Bladder Kidney	1565, 56-80	CC
Schiffman and Felton, 1990 ⁶⁶	OR=3.5	Colon	146, Ng	CC
Barrett et al., 2003 ⁶⁷	OR=1.97	Colorectal	2164, 45-80	CC
Tiemersma et al., 2004 ⁶⁸	No association	Colorectal	864, Ng	CC
Le Marchand et al., 2002 ⁶⁹	OR=8.8	Colorectal	1454, Ng	CC
Nowell et al., 2002 ⁷⁰	OR=4.36	Colorectal	460, 20-88	CC
Sinha et al., 2001 ⁷¹	OR=1.29	Colorectal	374, Ng	CC
Probst-Hensch et al., 1997 ⁷²	OR=2.2	Colorectal	976, 50-74	CC
Gerhardsson de Verdier et al., 1991 ⁷³	RR=2.8	Colorectal	1064, 42-81	CC
Terry et al., 2003 ⁷⁴	No Association No Association OR=2.4	Esophagus Gastric cardia Esophageal-squamous cell	1004, <80 1077, <80 982, <80	CC
Bosetti et al., 2002 ⁷⁵	OR=1.89	Laryngeal	1824, 31-79	CC
Sinha et al., 1998 ⁷⁵	OR=1.8	Lung	1216, 52-79	CC
Zhang et al., 1999 ⁷⁶	OR=2.2	Non-Hodgkin's lymphoma	88410, 48-74	P
Anderson et al., 2002 ⁷⁷	OR=2.19	Pancreatic	867, 20-65+	CC
Norrish et al., 1999 ⁷⁸	Positive trend	Prostate	787, Ng	CC
Nowell et al., 2004 ⁷⁹	OR=8.27	Prostate	923, Ng	CC
Murtaugh et al., 2004 ⁸⁰	OR=1.33	Rectal	2157, Ng	CC
Ward et al., 1997 ⁸¹	OR=2.4	Stomach	678, ~67-82	CC
	OR=2.0	Esophagus	645, ~67-82	

*Ng-Age range not clearly given.

Figure legends

Figure 1.

Structures of heterocyclic amine mutagens/carcinogens and creatine.

Heterocyclic amines are pyrosynthesized from creatine, amino acids and sugars.

Figure 2.

Formation of heterocyclic amines in beef patties after cooking to an internal temperature of 70°C at different frying pan temperatures. Error bars are the standard error of 4 or 5 replicate cooking experiments.

Figure 3.

Heterocyclic amines formed in a mixture representing beef, chicken breast and codfish amino acid, creatine, and glucose abundances that was dry-heated at 225°C for 30 min.

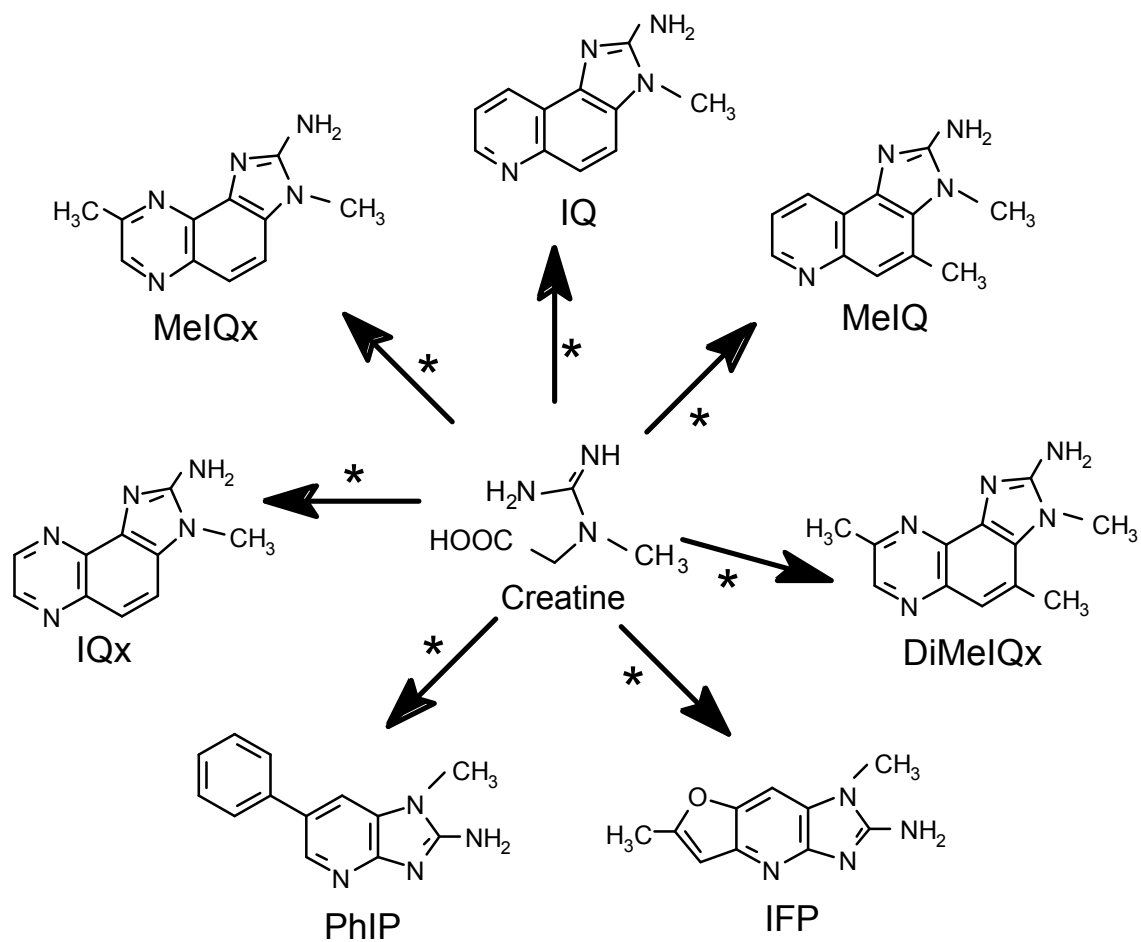


Figure 1

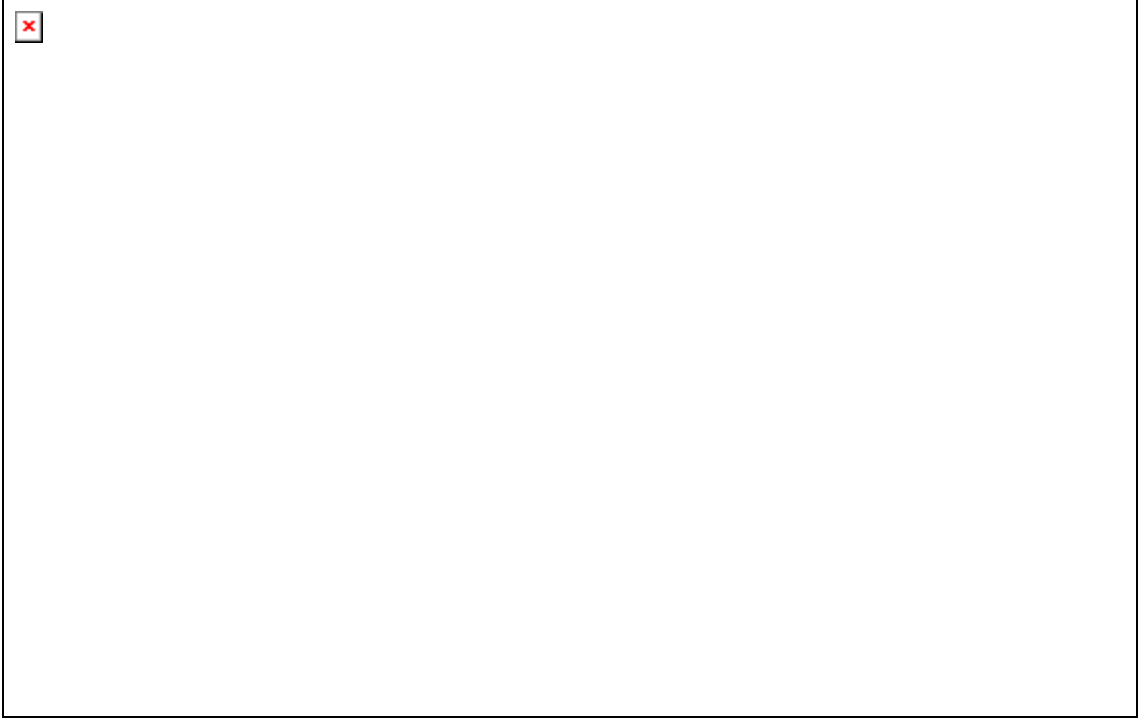


Figure 2

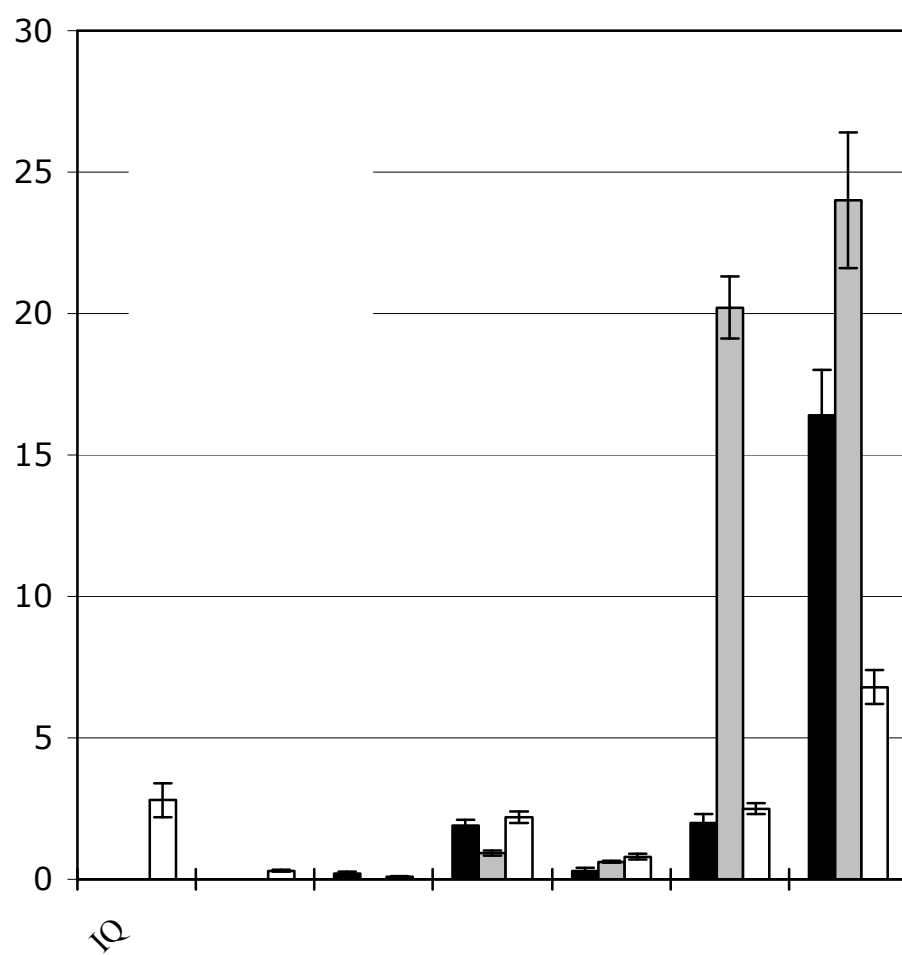


Figure 3